

higher neutralizing antibody and RBD219-N1-specific IgG titers than those formulated at the 1:8 ratio (Figure 2A-B). Consistent with the antibody responses, mice immunized with RBD219-N1/Alhydrogel® (1:25) were completely protected against lethal challenge with SARS-CoV, as indicated by undetectable infectious virus within the lungs and also a lack of morbidity and mortality (Figure 3). In contrast, infectious virus was recovered from the lungs of mice immunized with a 1:8 ratio of RBD219-N1/ Alhydrogel® (Groups 2 and 3) by day 5 p.i and one mouse from each group died. As expected, mice immunized with Alhydrogel® alone (Group 4) were not protected against lethal viral challenge and had detectable virus in the lungs resulting in the death of 2 mice on

day 4 p.i. While SARS-CoV S protein formulated on Alhydrogel® (Group 5) was also immunogenic and protective against SARS-CoV infection, histopathology examination of lung tissues consistently revealed enhanced infiltration of eosinophils (data not shown).

The difference in findings of the groups that received the 1:8 ratio compared to the 1:25 ratio was unexpected as at a 1:8 ratio of RBD219-N1/ Alhydrogel® the protein is completely adsorbed to the Alhydrogel® and it is not predicted that an excess of unbound recombinant protein, such as in the 1:25 ratio groups, should have a favorable impact on the vaccine efficacy. At this point, we consider these data to be inconclusive and are planning to repeat and expand the preclinical formulation studies in Year 5.

Aim 2.C. Execution of three successive process development runs at the 10 L scale.

We continued the optimization of our upstream and downstream processes, and proceeded with the three successive process development runs at 10 L scale. This approach using three independent process runs was used to ensure robustness of the process and yield unbiased results. The summary for the three runs (Table 1) shows the process was reproducible with a variance of less than 15%.

Table 1. Summary of the three successive process runs

Run #	Volume of FS (L)	Rough yield of FS (mg RBD/L of FS)	Final Concentration (mg RBD/mL)	Final yield (mg RBD/L of FS)	Process Recovery (%)
Run 1	5.8	424	1.80	250	54
Run 2	5.5	427	1.38	201	48
Run 3	5.9	424	1.68	228	54
Mean	5.7±0.2	425±1.6	1.62±0.22	226±25	52±3
%CV	3.5%	0.4%	13.4%	10.9%	7.0%

FS – indicates Fermentation Supernatant

Characterization of the purified RBD219-N1 from these three runs was performed using the developed assays (Aim 2.B.), including SDS-PAGE, Western Blot, Silver Staining, HPLC-PR, pH, A280 and Color and

Appearance. Based on the characterization, the three lots of RBD219-N1 were very similar with purity ≥95%. An accelerated stability study was conducted to monitor RBD219-N1 stability at different temperatures (4°C, room temperature and 37°C) over a month. The stability study results suggested the RBD219-N1 from three successive runs remained stable even at 37°C for a month.

Aim 3.A. Strategy for Manufacture of Drug Substance and Drug Product

Upon completion of the three reproducible process development runs, we arranged the technology transfer for cGMP Manufacture by providing a research clone for the generation of Master and Production Cell Banks. With the assistance of our quality assurance associates at the Sabin Vaccine Institute, batch production records were reviewed and finalized to ensure equitable procedures would be utilized by the authorized contract manufacturing organization for the final fermentation and purification of the RBD219-N1.

Aim 3.C. IND Regulatory Package Preparation and Submission

Preliminary work has begun on the preparation of a pre-IND briefing package by compiling information from the pre-clinical work for the CMC.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?**B.4 What opportunities for training and professional development has the project provided?**

Under the guidance of an experienced researcher, an under-represented minority high school student spent eight weeks learning and practicing the fundamental techniques in molecular biology and biochemistry while assisting with process development of a recombinant receptor-binding domain (rRBD) protein to prevent severe acute respiratory syndrome (SARS) caused by the SARS coronavirus (SARS CoV).

The student assisted with the development of downstream (purification) process thereby contributing to the optimization of a reproducible 10 L scale process for a stable rRBD-based vaccine. The student was also able to assist in supporting the three reproducible product development runs in preparation for future technology transfer to a cGMP manufacturing facility.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Du L, Jiang S. Middle East respiratory syndrome: current status and future prospects for vaccine development. <i>Expert Opin Biol Ther.</i> 2015;15(11):1647-51. PubMed PMID: 26414077; PubMed Central PMCID: PMC4636333.
Complete	Honda-Okubo Y, Barnard D, Ong CH, Peng BH, Tseng CT, Petrovsky N. Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology. <i>J Virol.</i> 2015 Mar;89(6):2995-3007. PubMed PMID: 25520500; PubMed Central PMCID: PMC4337527.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? Yes

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	HOTEZ, PETER J	PII		BA,PHD, MD	PD/PI	EFFORT	0	0			NA
	Y	Bottazzi, Maria Elena		BS,PHD	PD/PI	0		0			NA	
	Y	JIANG, SHIBO		MD,PHD	PD/PI	0		0	Fudan University	CHINA	NA	
	N	Tyagi Kundu, Rakhi		MS	Technician	0		0			NA	
	N	Agrawal, Anurodh		PhD	Technician	0		0			NA	
	N	Poongavanam selvamani, Mohan vivekanandan		PhD	Technician	0		0			NA	
	N	Chag, Shivali		MS	Non-Student Research Assistant	0		0			NA	
	Y	Tseng, Chien-Te K		PHD,MS	Co-Investigator	0		0			NA	
	N	Chan, Tehseng		MD, PhD	Co-Investigator	0		0			NA	
	Y	Du, Lanying		PHD	Co-Investigator	0		0			NA	
	Y	LUSTIGMAN, SARA		PHD	Co-Investigator	0		0			NA	
	N	Nino, Diane		BSci	Project Manager	0		0			NA	
	N	Peng, Bi-Hung		PhD	Faculty	0		0			NA	
	N	Han, Song	PhD	Research Associate III.	0	0			NA			
	N	Tao, Xinrong	Ph.D.	Research Associate	0	0			NA			

Glossary of acronyms:

S/K - Senior/Key
 DOB - Date of Birth
 Cal - Person Months (Calendar)
 Aca - Person Months (Academic)
 Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation
 SS - Supplement Support
 RE - Reentry Supplement
 DI - Diversity Supplement
 OT - Other
 NA - Not Applicable

D.2 PERSONNEL UPDATES**D.2.a Level of Effort**

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

Yes

File uploaded: Zhan NIH-biosketch_SARS.pdf

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: combined OS.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Zhan, Bin

eRA COMMONS USER NAME (agency login): eRA Commons User
Name

POSITION TITLE: Associate Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Fujian Medical College, Fuzhou, Fujian	MD	07/1983	Medicine
Chinese Academy of Preventive Medicine, Shanghai, Shanghai	MS	07/1989	Parasitology
University of Texas Medical Branch at Galveston, Galveston, Texas	Postdoctoral Fellow	12/1996	Molecular biology
Yale University, New Heaven, CT	Fellow	04/1997	Molecular Parasitology
Yale University, New Heaven, CT	Fellow	07/2000	Vaccine development

A. Personal Statement

Soon after I received my MD degree I decided to dedicate myself to fight against parasitic diseases and other infectious diseases after I realized millions people in my homeland China and other developing countries were suffering from these diseases. Since then, I have been involved in multidisciplinary studies of epidemiology, immunology, and molecular biology of malaria, leishmania, hookworm and other Neglected Tropical Diseases, especially with interest in the molecular basis of host-parasite interactions and targeting those crucial molecules for the parasitism as vaccine candidates. Particularly, I have focused my research on developing human hookworm vaccines since 2000 when the Human Hookworm Vaccine Initiative (HHVI) was funded by

Private Source

My research includes the molecular cloning of novel hookworm antigens by immunological and molecular biological approaches, characterizing their biological functions in the survival of hookworm in the host as well as evaluating their potential as protective antigens and/or vaccines. My work also involves the animal models for parasites and their life cycle maintenance and pre-clinical vaccine trials. From my work, more than 40 new hookworm antigens as well as their DNA have been identified and cloned from different species and stages of hookworms. Many of them have been characterized and tested for their vaccine potential. Some of these vaccine candidates are in process development and pilot manufacture for clinic trials. In addition to the vaccine development against hookworm, I also work for developing vaccines for other parasitic infections such as malaria, leishmaniasis, Ascariasis, Trichuriasis, Chagas disease, Onchocerciasis and SARS. In addition, For the past four years, I have involved in the design, cloning and expression of receptor-binding protein (RBD) of SARS coronavirus as a vaccine against SARS. I am intensively involved in developing recombinant protein expression in E. coli, yeast and baculovirus/insect cells and have a lot of experiences with these expression platforms. These researches have led to more than 90 publications in peer-reviewed journals.

Given my intensive experiences in protein expression and vaccine development against parasitic infections and SARS, I am confident that I can further contribute myself into the vaccine development against SARS/MERS using my expertise.

B. Positions and Honors

Positions and Employment

1983 - 1986	Assistant lecture, Fujian Medical College, Fuzhou
1989 - 1994	Assistant Professor, Chinese Academy of Preventive Medicine, Institute of Parasitic Diseases, Shanghai
1997 - 2000	Associate Professor, Chief of Pharmacology Department, Chinese Academy of Preventive Medicine, Institute of Parasitic Diseases, Shanghai
2000 - 2003	Assistant Research Professor, The George Washington University , Washington, DC
2004 - 2008	Associate Research Professor, The George Washington University , Washington, DC
2009 - 2010	Research Professor, The George Washington University , Washington , DC
2011 -	Associate Professor, Baylor College of Medicine, Houston, TX

C. Contribution to Science

- Hookworm antigen discovery and characterization:** In order to understand the strategies that hookworm utilizes to survive in the intestine of host and the interaction between hookworm and host, A great arsenal of hookworm secreted proteins or surface antigens were identified and cloned by immunoreaction with protective immune sera or other molecular biological approaches. The biological functions of these hookworm antigens were characterized. A total of more than 40 new hookworm antigens as well as their DNA has been identified and cloned from different species and stages of hookworms. Many of them have been characterized and tested for their vaccine potential.
 - Zhan B**, Hotez PJ, Wang Y, Hawdon JM. A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of proteases. *Mol Biochem Parasitol.* 2002 Apr 9;120(2):291-6.
 - Zhan B**, Liu Y, Badamchian M, Williamson A, Feng J, Loukas A, Hawdon JM, Hotez PJ. Molecular characterisation of the *Ancylostoma*-secreted protein family from the adult stage of *Ancylostoma caninum*. *Int J Parasitol.* 2003 Aug;33(9):897-907..
 - Zhan B**, Wang Y, Liu Y, Williamson A, Loukas A, Hawdon JM, Xue HC, Xiao SH, Hotez PJ. Ac-SAA-1, an immunodominant 16 kDa surface-associated antigen of infective larvae and adults of *Ancylostoma caninum*. *Int J Parasitol.* 2004 Aug;34(9):1037-45.
 - Jiang D, **Zhan B**, Mayor RS, Gillespie P, Keegan B, Bottazzi ME, Hotez P. Ac-AP-12, a novel factor Xa anticoagulant peptide from the esophageal glands of adult *Ancylostoma caninum*. *Mol Biochem Parasitol.* 2011 May;177(1):42-8.
- Vaccine development against hookworm and other parasite infections:** In order to evaluate the vaccine efficacy for those antigens I identified and cloned from hookworm, these antigens were expressed as recombinant proteins and immunized in dog or hamster animal models. The worm reduction was measured against challenge with hookworm infective larvae. Many of them showed effective protection against challenge and some of them have been chosen for clinical trials such as Na-ASP-2, Na-GST-1, Na-APR-1).
 - Mendez S, **Zhan B**, Goud G, Ghosh K, Dobardzic A, Wu W, Liu S, Deumic V, Dobardzic R, Liu Y, Bethony J, Hotez PJ. Effect of combining the larval antigens *Ancylostoma* secreted protein 2 (ASP-2) and metalloprotease 1 (MTP-1) in protecting hamsters against hookworm infection and disease caused by *Ancylostoma ceylanicum*. *Vaccine.* 2005 May 2;23(24):3123-30..
 - Zhan B**, Liu S, Perally S, Xue J, Fujiwara R, Brophy P, Xiao S, Liu Y, Feng J, Williamson A, Wang Y, Bueno LL, Mendez S, Goud G, Bethony JM, Hawdon JM, Loukas A, Jones K, Hotez PJ. Biochemical characterization and vaccine potential of a heme-binding glutathione transferase from the adult hookworm *Ancylostoma caninum*. *Infect Immun.* 2005 Oct;73(10):6903-11.
 - Zhan B**, Perally S, Brophy PM, Xue J, Goud G, Liu S, Deumic V, de Oliveira LM, Bethony J, Bottazzi ME, Jiang D, Gillespie P, Xiao SH, Gupta R, Loukas A, Ranjit N, Lustigman S, Oksov Y, Hotez P. Molecular cloning, biochemical characterization, and partial protective immunity of the heme-binding glutathione S-transferases from the human hookworm *Necator americanus*. *Infect Immun.* 2010 Apr;78(4):1552-63..
 - Hess JA, **Zhan B**, Bonne-Année S, Deckman JM, Bottazzi ME, Hotez PJ, Klei TR, Lustigman S, Abraham D. Vaccines to combat river blindness: expression, selection and formulation of vaccines against infection with *Onchocerca volvulus* in a mouse model. *Int J Parasitol.* 2014 Aug;44(9):637-46.

3. **Vaccine development against SARS/MERS:** Except for hookworm vaccine, I also devote myself into vaccine development against SARS and MERS. I am intensively involved in developing recombinant protein expression in *E. coli*, yeast and baculovirus/insect cells expression platforms to produce recombinant proteins for preclinical vaccine trials and immunodiagnosis.
 - a. Chen WH, Du L, Chag SM, Ma C, Tricoche N, Tao X, Seid CA, Hudspeth EM, Lustigman S, Tseng CT, Bottazzi ME, Hotez PJ, Zhan B, Jiang S. Yeast-expressed recombinant protein of the receptor-binding domain in SARS-CoV spike protein with deglycosylated forms as a SARS vaccine candidate. *Hum Vaccin Immunother*.
 - b. Hotez PJ, Bottazzi ME, Tseng CT, Zhan B, Lustigman S, Du L, Jiang S. Calling for rapid development of a safe and effective MERS vaccine. *Microbes Infect*. 2014 Jul;16(7):529-31. PubMed PMID: [24931059](https://pubmed.ncbi.nlm.nih.gov/24931059/).
 - c. Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, Jones K, Zhan B, Hotez PJ. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. *Expert Rev Vaccines*. 2012 Dec;11(12):1405-13. PubMed PMID: [23252385](https://pubmed.ncbi.nlm.nih.gov/23252385/); PubMed Central PMCID: [PMC3586247](https://pubmed.ncbi.nlm.nih.gov/PMC3586247/)
 - d. **Zhan B**, Ajmera R, Geiger SM, Gonçalves MT, Liu Z, Wei J, Wilkins PP, Fujiwara R, Gazzinelli Guimaraes PH, Bottazzi ME, Hotez P. Identification of immunodominant antigens for the laboratory diagnosis of toxocariasis. *Trop Med Int Health*. 2015 Dec;20(12):1787-1796.
4. **Hookworm animal models for vaccine and drug trials:** Many efforts have devoted to maintain hookworms in dog (*Ancylostoma caninum*) or in hamsters (*Necator americanus* and *A. ceylanicum*) as models for testing vaccine and drug trials. These models have led to several successful vaccine and drug products.
 - a. Jian X, Shu-Hua X, Hui-Qing Q, Sen L, Hotez P, Bing-Gui S, Hai-Chou X, Tie-Hua L, **Bin Z**. *Necator americanus*: maintenance through one hundred generations in golden hamsters (*Mesocricetus auratus*). II. Morphological development of the adult and its comparison with humans. *Exp Parasitol*. 2003 Nov-Dec;105(3-4):192-200.
 - b. Xiao S, **Zhan B**, Xue J, Goud GN, Loukas A, Liu Y, Williamson A, Liu S, Deumic V, Hotez P. The evaluation of recombinant hookworm antigens as vaccines in hamsters (*Mesocricetus auratus*) challenged with human hookworm, *Necator americanus*. *Exp Parasitol*. 2008 Jan;118(1):32-40.
 - c. Xue J, **Zhan B**, Guo J, He N, Qiang HQ, Hotez P, Xiao SH. Acquired hookworm immunity in the golden hamster (*Mesocricetus auratus*) elicited by living *Necator americanus* third-stage infective larvae. *Exp Parasitol*. 2012 Jan;130(1):6-12.
 - d. Hu Y, **Zhan B**, Keegan B, Yiu YY, Miller MM, Jones K, Aroian RV. Mechanistic and single-dose in vivo therapeutic studies of Cry5B anthelmintic action against hookworms. *PLoS Negl Trop Dis*. 2012;6(11):e1900.
5. **Immunomodulation of nematode-secreted proteins:** Parasitic nematodes secrete a lot of proteins with roles in immunomodulating host immune response as a survival strategy, simultaneously, infected hosts get benefits from these proteins in balancing their immune system. Some of these nematode-secreted proteins were identified and used for treatment of allergic or autoimmune diseases.
 - a. **Zhan B**, Badamchian M, Meihua B, Ashcom J, Feng J, Hawdon J, Shuhua X, Hotez PJ. Molecular cloning and purification of Ac-TMP, a developmentally regulated putative tissue inhibitor of metalloprotease released in relative abundance by adult *Ancylostoma* hookworms. *Am J Trop Med Hyg*. 2002 Mar;66(3):238-44.
 - b. **Zhan B**, Gupta R, Wong SP, Bier S, Jiang D, Goud G, Hotez P. Molecular cloning and characterization of Ac-TMP-2, a tissue inhibitor of metalloproteinase secreted by adult *Ancylostoma caninum*. *Mol Biochem Parasitol*. 2008 Dec;162(2):142-8.
 - c. **Zhan B**, Santiago H, Keegan B, Gillespie P, Xue J, Bethony J, de Oliveira LM, Jiang D, Diemert D, Xiao SH, Jones K, Feng X, Hotez PJ, Bottazzi ME. Fusion of Na-ASP-2 with human immunoglobulin Fc γ abrogates histamine release from basophils sensitized with anti-Na-ASP-2 IgE. *Parasite Immunol*. 2012 Aug-Sep;34(8-9):404-11.
 - d. Yang X, Yang Y, Wang Y, **Zhan B**, Gu Y, Cheng Y, Zhu X. Excretory/secretory products from *Trichinella spiralis* adult worms ameliorate DSS-induced colitis in mice. *PLoS One*. 2014;9(5):e96454.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/bin.zhan.1/bibliography/43438454/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

2015/01/01-2017/12/31

0000, [Private Source] Peter Hotez (PI)

Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease
The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.
Role: KP

2012/07/31-2017/12/31

0000, Department of Health and Human Services / Texas A&M Univ; Peter Hotez (PI)

Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

Role: OP

2013/01/15-2017/12/31

1R01AI105431-01 , NIH via New York Blood Center; Sara Lustigman (PI)

Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Role: Co-Investigator

2014/01/01-2017/12/31

0000, [Private Source] Hotez/Bottazzi (PI)

Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Role: Co-Investigator

2013/09/01-2017/08/31

0000, [Private Source] Peter Hotez (PI)

Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate Ascaris antigen and a Trichuris antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

Role: Co-Investigator

02/03/2015-01/31/2020

1 R01 CA183984-01A1, NIH/NCI; Qizhi Cathy Yao

A Novel miR-198 replacement therapy for pancreatic cancer

The main goal of this project is Mesothelin-targeted nanoparticle delivery of gemcitabine for pancreatic cancer

Role: Co-Investigator

Completed Research Support

2009/08/25-2015/07/31

1 R01A1078314 03 , NIH/NIAID (Sub-award from New York Blood Center); Sara Lustigman (PI)

The development of a recombinant vaccine against human onchocerciasis

The major goal of this subcontract is clone, express, characterize and optimize the expression of the eight selected *Onchocerca* vaccine candidate antigens (rOvAgs) using the yeast *Pichia* eukaryotic system.

Role: CPI

2014/08/01-2015/07/31

0000, Private Source Zhan/Cheolho (PI)

Anti-filarial vaccine discovery through transcriptome analysis of *Brugia pahangi*

The overarching goal of this one-year project is to identify filarial transmission-blocking vaccine candidates by deep sequencing analysis of the transcriptome of *Brugia pahangi* L3 in mosquito.

Role: CPI

2011/08/01-2015/04/30

1016395, Private Source Peter Hotez (PI)

Human Hookworm Vaccine Initiative 3

The project purpose is to provide proof-of-principle that vaccination with two adult-stage hookworm antigens will reduce the burden of infection caused by *Necator americanus*.

Role: KP

2010/08/01-2014/04/30

2R01AI056189 - 07 , NIH; Ravi Aroian (PI)

B. thuringiensis Crystal Proteins as Powerful Next-Generation Anthelmintics

The major goal of this subcontract is to test the effects of different formulated Cry5B against hookworm using *Ancylostoma ceylanicum*/hamster model

Role: CPI

2011/01/01-2013/12/31

0000, Dutch Government ; Peter Hotez (PI)

Product Development Support of the Human Hookworm Vaccine

The ultimate goal of the project is to conduct Phase 1 studies to assess the safety and immunogenicity of the Na-GST-1 and Na-APR-1 hookworm antigens in both adults and children.

Role: KP

2012/01/01-2012/09/15

HHSN272201001148P, NIH; Michael Bukrinsky (PI)

Immunogenicity and *Leishmania* Vaccine potential of Sand Fly Saliva in Humans

The findings obtained from this proposal will lead to the next steps in early process development of vaccine candidates and demonstrate that proteins can be produced by fermentation and successfully purified and formulated in a consistent and reproducible manner. The ultimate goal is that these basic processes and procedures will be used in downstream cGMP manufacture, GLP toxicology studies and ultimately in the preparation of an Investigational New Drug (IND) application to be submitted to the US Food and Drug Administration in order to commence First-in Human trials.

Role: KP

**OTHER SUPPORT
AT BAYLOR COLLEGE OF MEDICINE**

HOTEZ, PETER J.**New grants awarded:**

Instituto Carlos Slim de la Salud 11/1/15 – 10/31/18

Private Source 1/1/15 – 12/31/17

Grant ended:

Dutch Government grant

CURRENT

Hotez (PI) 04/20/2012 – 12/31/2016

EFFORT

Private Source \$229,534

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

5R01AI098775-02 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

EFFORT

Sponsor: National Institutes of Health \$782,695

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Bottazzi (Center Director, Consultant) 11/01/2012 – 08/31/2016

EFFORT

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$145,090

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

Role: Instructor

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017

EFFORT

Sponsor: Private Source \$275,435

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

Hotez (PI) 01/01/2014 – 12/31/2016

EFFORT

Sponsor: University of Malaya \$250,000

Title: Malaysian Neglected Tropical Disease Initiative

Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

Hotez (PI) 01/01/2014 – 12/31/2017

EFFORT

Sponsor: Private Source \$160,000

Title: West Nile Virus vaccine development

Main goal is to support West Nile Virus vaccine development.

Hotez/Bottazzi (MPI) 01/01/2014 – 07/31/2017

EFFORT

Sponsor: Private Source \$104,620

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Bottazzi (PI) 07/01/2012 – 10/31/2018

EFFORT

Sponsor: Instituto Carlos Slim de la Salud \$887,044

Title: Slim Initiative for Antipoverty Vaccine Development (Chagas)

The main goal of this project is to bring a Chagas vaccine candidate, Tc24, to a stage of development where pre-IND packages are submitted to the FDA and COFEPRIS. Also an overarching PRINCIPLE is to build human and infrastructure capacity for vaccine development, manufacture, regulatory science, and first in humans clinical testing in Mexico. The major DELIVERABLE is a developed process for refolding TSA-1.

Hotez (PI) 11/1/2014 – 10/31/2016

EFFORT

Sponsor: Private Source

Title: Adjuvant Technologies to Advance Chagas Disease Vaccine Development \$323,651

The overall goal of this project is to develop a new vaccine formulation for Chagas disease consisting of a promising protein-based antigen (Tc24) formulated with a novel TLR4 agonist adjuvant, E6020, which is designed to skew the immune response toward a T_H1 bias and the generation of cytotoxic T cells.

Hotez (PI) 01/01/2015 – 12/31/2017

EFFORT

Sponsor: Private Source

\$582,355

Title: Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease

Main Goal: The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.

OTHER SUPPORT - BOTTAZZI, MARIA ELENA**New grants awarded:**

Instituto Carlos Slim de la Salud 11/1/15 – 10/31/18

Private Source 1/1/15 – 12/31/17

ACTIVE

Hotez (PI) 04/20/2012 – 12/31/2016

Private Source \$229,319

EFFORT

Accelerating the development and testing of a therapeutic Chagas vaccine

The main goal of this project is to accelerate the early development of a vaccine for a major neglected tropical disease affecting the Amazon region and Latin America – Chagas disease.

Role: Director of Product Development

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

Sponsor: National Institutes of Health \$782,695

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

EFFORT

Bottazzi (Center Director, Consultant) 11/01/2012 – 08/31/2016

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$145,090

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

EFFORT

1R01AI105431-01 Lustigman (PI) 01/15/2013 – 12/31/2017

Sponsor: NIH via New York Blood Center \$137,860

Title: Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Role: Sub-PI

EFFORT

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017

Sponsor: Private Source \$275,435

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

EFFORT

HOOKVAC Bottazzi (PI) 10/1/2013 – 9/30/2017

Sponsor: European Union via sub from (AIGHD) \$13,377

Title: Developing and Testing a novel, low-cost, effective HOOKworm VACcine to Control Human Hookworm Infection in endemic countries

Major goals of the project are to perform technology transfer of processes for fermentation purification and analytical testing of the human hookworm vaccine.

EFFORT

Hotez (PI) 01/01/2014 – 12/31/2016

Sponsor: University of Malaya \$250,000

Title: Malaysian Neglected Tropical Disease Initiative

Major role of the project is to train and build capacity for Malaysian scientists in the area of vaccine biotechnology.

Role: Co-I

EFFORT

Hotez (PI)	01/01/2014 – 12/31/2017		EFFORT
Sponsor: Private Source		\$160,000	
Title: West Nile Virus vaccine development			
Main goal is to support West Nile Virus vaccine development.			
Role: Co-I			
Hotez/Bottazzi (MPI)	01/01/2014 – 07/31/2017		EFFORT
Sponsor: Private Source		\$104,620	
Title: Hookworm Vaccine Discovery Program			
The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.			
Bottazzi (Sub-PI)	09/1/2014 – 08/31/2016		EFFORT
Sponsor: Private Source		\$438,396	
Title: Adjuvant Technologies to Advance Chagas Disease Vaccine Development			
The overall goal of this project is to develop a new vaccine formulation for Chagas disease consisting of a promising protein-based antigen (Tc24) formulated with a novel TLR4 agonist adjuvant, E6020, which is designed to skew the immune response toward a T _H 1 bias and the generation of cytotoxic T cells.			
Bottazzi (PI)	07/01/2012 – 10/31/2018		EFFORT
Sponsor: Instituto Carlos Slim de la Salud		\$887,044	
Title: Slim Initiative for Antipoverty Vaccine Development (Chagas)			
The main goal of this project is to bring a Chagas vaccine candidate, Tc24, to a stage of development where pre-IND packages are submitted to the FDA and COFEPRIS. Also an overarching PRINCIPLE is to build human and infrastructure capacity for vaccine development, manufacture, regulatory science, and first in humans clinical testing in Mexico. The major DELIVERABLE is a developed process for refolding TSA-1.			
Hotez (PI)	01/01/2015 – 12/31/2017		EFFORT
Sponsor: Private Source		\$582,355	
Title: Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease			
Main Goal: The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.			

OTHER SUPPORT – ZHAN, BIN

Private Source

1/1/15 – 12/31/17

ACTIVE

Bottazzi (Center Director, Consultant) 11/01/2012 – 08/31/2016

EFFORT

Sponsor: Department of Health and Human Services / Texas A&M Univ. \$145,090

Title: Centers for Innovation in Advanced Development and Manufacturing

The major goal of this project is to advance education and training for professionals in the area of vaccine biotechnology and product development.

Role: PT Instructor

1R01AI105431-01 Lustigman (PI) 01/15/2013 – 12/31/2017

EFFORT

Sponsor: NIH via New York Blood Center \$137,860

Title: Development of a novel adjuvant for vaccine sparring

Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

Role: Co-Investigator

R01AI098775-01 Hotez/Bottazzi/Jiang (MPI) 05/04/2012 – 04/30/2017

EFFORT

Sponsor: National Institutes of Health \$782,695

Title: RBD Recombinant Protein-based SARS Vaccine for Biodefence

The main goal of this project is to develop, test and manufacture a novel recombinant SARS vaccine.

Role: Co-Investigator

Hotez/Bottazzi (MPI) 08/01/2013 – 07/31/2017

EFFORT

Sponsor: Private Source \$275,435

Title: Multivalent Anthelmintic Vaccine Discovery Program

The overarching goal of this four year project is to advance the development of a lead candidate *Ascaris* antigen and a *Trichuris* antigen, either or both of which ultimately could be formulated with the Human Hookworm Vaccine now under development by the Sabin PDP.

Role: Co-Investigator

Hotez/Bottazzi (MPI) 01/01/2014 – 07/31/2017

EFFORT

Sponsor: Private Source \$104,620

Title: Hookworm Vaccine Discovery Program

The overarching goal of this four year project is to discover new candidate antigens for the development of a hookworm vaccine to complement the Human Hookworm Vaccine now under development by the Sabin PDP.

Role: Co-Investigator

Hotez (PI) 01/01/2015 – 12/31/2017

EFFORT

Sponsor: Private Source \$582,355

Title: Chagas Vaccine Initiative: Accelerated Development of the First Therapeutic Vaccine for Chagas Disease

Main Goal: The goal of this project is to develop a process for the production of a second Chagas disease vaccine antigen, co-formulate it with the Tc24 antigen, and conduct pre-clinical immunogenicity and efficacy studies.

Role: Director, Molecular Biology

Yao (PI) 02/03/2015-01/31/2020

EFFORT

Sponsor: NIH/NCI \$299,000

Title: A Novel miR-198 replacement therapy for pancreatic cancer

The main goal of this project is Mesothelin-targeted nanoparticle delivery of gemcitabine for pancreatic cancer.

Role: Co-Investigator

**OTHER SUPPORT
AT NEW YORK BLOOD CENTER**

Changes for Dr. Lustigman:

NIH (b)(4) were submitted
OPP1083910; OPP1099849; 1R01AI078314-01A2; OPP1086618; 1R56 1AI101372-01A1: ended
No changes in all other four previously funded projects

SARA LUSTIGMAN

ACTIVE

1. 1R01AI105431-01 (PI: S. Lustigman) 1/2013 – 12/2017
NIH/NIAID \$ \$817,780 annual direct (including two subcontracts) (b)(6)

Development of a novel adjuvant for vaccine sparring

Adjuvants are integrated into vaccines to insure their effectiveness and to support antigen sparing. Currently, alum is the only adjuvant licensed in the U.S., but it has had limited effectiveness when used with commercial flu vaccines. Our objective is to produce a highly effective and safe rASP-1 adjuvanted flu vaccine in a simple aqueous formulation that requires a minimal antigen quantity per dose. By enhancing vaccine efficacy in this way, we can effectively increase the number of vaccine doses available.

2. OPP1017584 (PI:J Sakanari; Co-PI: S. Lustigman) 11/2012 – 10/2017
Bill & Melinda Gates Foundation \$ 216,428 annual direct (subcontract) (b)(6)

Developing a macrofilaricidal drug for onchocerciasis using Anacor's novel oxaborole technology

A collaborative research effort between the University of California San Francisco Sandler Center, Anacor Pharmaceuticals and LFKRI of the NYBC to discover new drug therapies for the treatment of river blindness (onchocerciasis). The collaboration's goal is to identify a novel, potent macrofilaricidal drug candidate that is capable of killing adult worms.

3. OPP1083910 (PI: T. Nutman; Co-PI: Lustigman) 8/2013 – 3/2016
Bill & Melinda Gates Foundation \$76,834 annual direct (Subcontract) (b)(6)

Rapid identification of individuals with viable adult female worms of Onchocerca volvulus: a means to the end

To identify host- and parasite-specific biomarker(s) present in human subjects with viable adult females of *Onchocerca volvulus* and to develop and configure rapid point of care methods to detect (or sense) these biomarkers; a necessary step in the progress towards elimination of onchocerciasis.

PENDING

(b)(4)

Pending Support

OVERLAP

None; effort commitments will be adjusted as necessary if pending proposals are awarded.

Changes for Drs. Shibo Jiang and Lanying Du:

SHIBO JIANG

ACTIVE

5R01 AI098775-04 Hotez, Bottazzi, Jiang (PIs)
05/01/2012 – 04/30/2017

EFFORT

NIH/NIAID
RBD recombinant protein-based SARS vaccine for biodefense
Role: MPI
Overlap: None

ACTIVE

5R21 AI111152-02 (Du) 08/05/2014 – 07/31/2016

EFFORT

NIH/NIAID
B. subtilis spore-delivered M2e-FP-based mucosal universal influenza vaccines
Role: Co-Investigator
Overlap: None

PENDING

Pending Support

LANYING DU

ACTIVE

5R21 AI111152-02 (Du) 08/05/2014 – 07/31/2016

EFFORT

NIH/NIAID
B. subtilis spore-delivered M2e-FP-based mucosal universal influenza vaccines
Role: PI
Overlap: None

PENDING

Pending Support

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE Not Applicable
F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM NOTHING TO REPORT
F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS F.3.a Human Subjects No Change
F.3.b Vertebrate Animals No Change
F.3.c Biohazards No Change
F.3.d Select Agents File uploaded: utmb select agent letter.pdf



**Environmental Health & Safety
Biological & Chemical Safety Program**
Materials Management Building, 2.112
301 University Blvd.
Galveston, Texas 77555-1111
O 409.772.1781 F 409.772.8921

March 14, 2016

Dear Colleague:

The University of Texas Medical Branch at Galveston (UTMB) is a select agent registered entity with the U.S. Health and Human Services, Centers for Disease Control and Prevention Division of Select Agents and Toxins (CDC/DSAT) and U. S. Department of Agriculture, Animal and Plant Health Inspection Service, (USDA/APHIS) National Select Agent Program. The University has been inspected by the CDC/DSAT and USDA/APHIS National Select Agent Program for use of HHS Select Agents, Overlap Select Agents and USDA Select Agents.

Per the requirements of 42 CFR 73 the original certificate of registration was issued October 19, 2004, renewal was granted on April 9, 2007, April 1, 2010 and on March 21, 2012 for use of select agents at BioSafety Levels 2, 3, and 4 and Animal BioSafety Levels 3 and 4. Inspection by CDC/DSAT and USDA/APHIS occurred January 12th to 16th 2015, for the current renewal cycle and approval was granted on March 18, 2015 for three years. The Keiller BSL2, BSL3 East and BSL3 West labs and the ACL3, Robert E. Shope BSL4/ABSL4 and irradiator, the Galveston National Laboratory BSL2 and BSL3, ABSL3, BSL3 Enhanced and BSL4/ABSL4, ABSL4 Aerobiology and irradiator facilities are all select agent registered.

The University has been registered with Health and Human Services, Centers for Disease Control and Prevention as a select agent facility since 1997.

A copy of the registration form is attached. The registration number has been redacted for security purposes.

Please feel free to contact me should you require additional information.

Sincerely,

A handwritten signature in black ink that reads "Domenica Zimmerman". The signature is written in a cursive, flowing style.

Domenica Zimmerman
BioSafety Officer
Alternate Responsible Official
UTMB Select Agent Program

Certificate of Registration

Entity Name: University of Texas Medical Branch
Address: 301 University Boulevard
Galveston, TX 77555

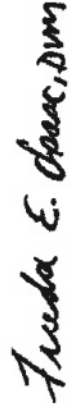
Registration #:
Effective Date: March 18, 2015
Expiration Date: March 18, 2018

Responsible Official: Michael Shriner
Alternate Responsible Official(s): Carlos Escobar, Danny Jacobs, Scott Weaver, Domenica Zimmerman

Based on information provided to the CDC Select Agent Program and the APHIS Agriculture Select Agent Services, the above named entity is authorized to possess, use, and transfer select agents and toxins under the conditions specified in the entity registration application, in accordance with 42 CFR Part 73, 9 CFR Part 121, and 7 CFR Part 331.



Robbin S. Weyant, Director
Division of Select Agents and Toxins
Centers for Disease Control and Prevention



Freeda E. Isaac, DVM, National Director
Agriculture Select Agent Services
Animal and Plant Health Inspection Service



G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

File(s) uploaded:
utmb select agent.pdf

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS**G.4.a Does the project involve human subjects?**

Yes

Is the research exempt from Federal regulations?

Yes

Exemption number(s) E4

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Report Attached: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

G.4.c ClinicalTrials.gov**Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?**

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT**Are there personnel on this project who are newly involved in the design or conduct of human subjects research?**

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS**Does this project involve vertebrate animals?**

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: BAYLOR COLLEGE OF MEDICINE	051113330	TX-009	BAYLOR COLLEGE OF MEDICINE ONE BAYLOR PLAZA HOUSTON TX 770303411
New York Blood Center	073271827	NY-014	310 East 67 Street New York NY 100656275
The University of Texas Medical Branch	800771149	TX-014	301 University Boulevard Galveston TX 775550156
Texas Childrens Hospital	074615394	TX-009	1102 Bates Street Houston TX 770302399

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No



Environmental Health & Safety
Biological & Chemical Safety Program
Materials Management Building, 2.112
301 University Blvd.
Galveston, Texas 77555-1111
O 409.772.1781 F 409.772.8921

February 11, 2013

To Whom It May Concern

The University of Texas Medical Branch at Galveston (UTMB) is a select agent registered entity with the U.S. Health and Human Services, Centers for Disease Control and Prevention Division of Select Agents and Toxins (CDC/DSAT) and U. S. Department of Agriculture, Animal and Plant Health Inspection Service, (USDA/APHIS) National Select Agent Program. The University has been inspected by the CDC/DSAT and USDA/APHIS National Select Agent Program for use of HHS Select Agents and Toxins, Overlap Select Agents and Toxins and USDA Select Agents and Toxins.

Per the requirements of 42 CFR 73 the original certificate of registration was issued October 19, 2004, renewal was granted on April 9, 2007 and again on April 1, 2010 for use of select agents at BioSafety Levels 2, 3, and 4 and Animal BioSafety Levels 3 and 4. Inspection by CDC/DSAT and USDA/APHIS occurred January 9th to 20th 2012, for the current renewal cycle and approval was granted on March 21, 2012 for three years. The University has been registered with Health and Human Services, Centers for Disease Control and Prevention as a select agent facility since 1997. The University has a Responsible Official and four Alternate Responsible Officials.

Attached please find a copy of the University Of Texas Medical Branch certificate of registration of the possession, use and transfer of select agents and toxins. The registration number has been redacted for security purposes. The registration number will be provided at the time of an official CDC/USDA Form 2 transfer of select agents.

Please feel free to contact me should you require additional information.

Sincerely,

A handwritten signature in cursive script that reads "Domenica Zimmerman".

Domenica Zimmerman
BioSafety Officer
Alternate Responsible Official
UTMB Select Agent Program

Certificate of Registration

Entity Name: **University of Texas Medical Branch**
Address: **301 University Boulevard**
Galveston, TX 77555-0633

Registration #: **March 21, 2012**
Effective Date: **March 21, 2015**
Expiration Date: **March 21, 2015**



Responsible Official: **Michael Shriner**
Alternate Responsible Official(s): **Carlos Escobar, Amy Goebel, Scott Weayer, Domenica Zimmerman**

Based on information provided to the CDC Select Agent Program and the APIS, I am authorizing the above-named entity to possess, use, and transfer select agent **[redacted]** as specified in the entity registration application, in accordance with 42 CFR part 73, 9 CFR part 121, and 7 CFR part 331.

[Signature]

Robbin S. Weyant, Director
Select Agent Program
Centers for Disease Control and Prevention



[Signature]

Freda E. Isaac, DVM, Director
Select Agent Program
Veterinary Services

[Signature]

Charles L. Divan, Branch Chief
Select Agent Program
Plant Protection and Quarantine



Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 154039

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Domestic

NIH Defined Phase III Clinical Trial: No

Study Title: RBD recombinant protein-based SARS vaccine for biodefense-PROTOCOL-001

Planned Enrollment

Comments: This project is not required to enroll subjects. The research has exemption approval and only involves the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens.

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/Not Reported	Female	Male	Unknown/Not Reported	Female	Male	Unknown/Not Reported	
American Indian/Alaska Native	0	0		0	0					0
Asian	0	0		0	0					0
Native Hawaiian or Other Pacific Islander	0	0		0	0					0
Black or African American	0	0		0	0					0
White	0	0		0	0					0
More than One Race	0	0		0	0					0
Unknown or Not Reported										
Total	0	0		0	0					0

Cumulative Enrollment

NOTE: No cumulative inclusion enrollment data exists in the previous inclusion format or modified format. Although prompted to do so, the PD/PI did not enter information in the modified format. No data can be provided.



Grant Number: 5R01AI098775-02

Principal Investigator(s):

Maria Elena Bottazzi
PETER J HOTEZ (contact), PHD
SHIBO JIANG, MD

Project Title: RBD recombinant protein-based SARS vaccine for biodefense

Leanne Brooks Scott
Business Official
One Baylor Plaza, BCM320A
Houston, TX 770303411

Award e-mailed to: bcmaward@bcm.edu

Budget Period: 05/01/2013 – 04/30/2014

Project Period: 05/04/2012 – 04/30/2017

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of \$1,085,321 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to BAYLOR COLLEGE OF MEDICINE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI098775. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with 42 CFR Part 50 Subpart F. Subsequent to the compliance date of the 2011 revised FCOI regulation (i.e., on or before August 24, 2012), Awardees must be in compliance with all aspects of the 2011 revised regulation; until then, Awardees must comply with the 1995 regulation. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website <http://grants.nih.gov/grants/policy/coi/> for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Michael W. Fato

Additional information follows

SECTION I – AWARD DATA – 5R01AI098775-02

Award Calculation (U.S. Dollars)

Federal Direct Costs	\$955,528
Federal F&A Costs	\$129,793
Approved Budget	\$1,085,321
Federal Share	\$1,085,321
TOTAL FEDERAL AWARD AMOUNT	\$1,085,321
 AMOUNT OF THIS ACTION (FEDERAL SHARE)	 \$1,085,321

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
2	\$1,085,321	\$1,085,321
3	\$1,134,359	\$1,134,359
4	\$1,165,726	\$1,165,726
5	\$1,165,855	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Number: 93.855
EIN: 1741613878A1
Document Number: RAI098775A
Fiscal Year: 2013

IC	CAN	2013	2014	2015	2016
AI	8472315	\$1,085,321	\$1,134,359	\$1,165,726	\$1,165,855

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:

PCC: M51C B / **OC:** 414E / **Released:** [Redacted] 05/14/2013
Award Processed: 05/15/2013 12:14:44 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI098775-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at <http://grants.nih.gov/grants/policy/awardconditions.htm>

SECTION III – TERMS AND CONDITIONS – 5R01AI098775-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Award.
- b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
- c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- d. The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at 'http://grants.nih.gov/grants/policy/awardconditions.htm' for certain references cited above.)

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the Central Contractor Registration. Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See <http://grants.nih.gov/grants/policy/awardconditions.htm> for the full NIH award term implementing this requirement and other additional information.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see <http://grants.nih.gov/grants/policy/awardconditions.htm> for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: <http://publicaccess.nih.gov/>.

Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI098775-02

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

This award is issued in accordance with the FY2013 NIH fiscal policies.

RESTRICTION: Under governing PHS Policy, Federal funds administered by the Public Health Service (PHS) shall not be expended for research involving live vertebrate animals without prior approval by the Office of Laboratory Animal Welfare (OLAW) of an Assurance to comply with the PHS Policy on Humane Care and Use of Laboratory Animals and the project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The present award is being made without currently valid verification of IACUC approval for the portion of this project being completed in CHINA with the following restriction: No activities that involve live vertebrate animals may be conducted at Frontier Biosciences located in CHINA pending acceptance by the NIH awarding component of verification of IACUC approval. The Program Officer has approved the funding of this application without the portion of Frontier Biosciences located in CHINA in year 05 as the project is viable without it. No funds may be expended for the foreign site pending the resolution of internal administrative issues. Once these issues have been resolved, this award may be revised to include the study originally planned for the foreign site. Failure to comply with this special condition can result in suspension and/or termination of this award, withholding of support, audit disallowances, and/or other appropriate action.

This award includes funds awarded for consortium activity with NY Blood Center. Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2012/nihgps_ch15.htm#_Toc271265264.

This award includes funds awarded for consortium activity with the University of Texas Medical Branch. Consortia are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps_2012/nihgps_ch15.htm#_Toc271265264.

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at <http://www.selectagents.gov/Regulations.html>) must complete registration with CDC (or APHIS,

depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS

Select Agents:

Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (<http://www.selectagents.gov/Regulations.html>).

Highly Pathogenic Agent:

NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (<http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm>). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.

When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- o A list of the new and/or additional Agent(s) that will be studied;
- o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Vandhana Khurana

Email: khuranav@mail.nih.gov **Phone:** 301-496-7075 **Fax:** 301-493-0597

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI098775-02

INSTITUTION: BAYLOR COLLEGE OF MEDICINE

Budget	Year 2	Year 3	Year 4	Year 5
TOTAL FEDERAL DC	\$955,528	\$899,886	\$782,695	\$768,645
TOTAL FEDERAL F&A	\$129,793	\$234,473	\$383,031	\$397,210
TOTAL COST	\$1,085,321	\$1,134,359	\$1,165,726	\$1,165,855

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	57.3%	57.3%	57.3%	57.3%
F&A Cost Base 1	\$226,514	\$409,202	\$668,466	\$693,211
F&A Costs 1	\$129,793	\$234,473	\$383,031	\$397,210

A. RPPR COVER PAGE

Project Title: RBD recombinant protein-based SARS vaccine for biodefense	
Grant Number: 5R01AI098775-02	Project/Grant Period: 05/04/2012 - 04/30/2017
Reporting Period: 05/04/2012 - 04/30/2013	Requested Budget Period: 05/01/2013 - 04/30/2014
Report Term Frequency: Annual	Date Submitted: 03/07/2013
Program Director/Principal Investigator Information: PETER J HOTEZ , MD PHD BA Phone number: 832-824-0502 Email: hotez@bcm.edu	Recipient Organization: BAYLOR COLLEGE OF MEDICINE BAYLOR COLLEGE OF MEDICINE 1 BAYLOR PLAZA MS-310 HOUSTON, TX 770303411 DUNS: 051113330 EIN: 1741613878A1 RECIPIENT ID: 35116-N1
Change of Contact PD/PI: N/A	
Administrative Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu	Signing Official: LEANNE BROOKS SCOTT One Baylor Plaza Houston, TX 77030 Phone number: 713-798-6978 Email: spo@bcm.edu
Human Subjects: Yes HS Exempt: Yes Exemption Number: E4 Phase III Clinical Trial:	Vertebrate Animals: Yes
hESC: No	Inventions/Patents: No

B. ACCOMPLISHMENTS**B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?**

The major goals of the project are: Specific Aim 1: Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate (Timeline Year 1-3). Specific Aim 2: Process development, characterization, formulation and stability profiling (Timeline Year 2-4) and Specific Aim 3: Technology transfer, cGMP Manufacture, GLP toxicology and IND Preparation (Timeline Year 4-5).

As proposed, for this reporting period activities related to Specific Aim 1 (Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate) were initiated. Specifically, we have achieved 50% completion of the activities related to the sub-specific aims 1.A. Feasibility of scalable expression, 1.B. Antigenicity and functionality and 1.C. Immunogenicity. For sub-specific aim 1.D. Efficacy, 33.3% of this activity has been completed. The goals will not change for the next reporting period and no significant changes in approach or methods are envisioned.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITITES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For Year 2 activities will continue for Specific Aim 1 and new activities under Specific Aim 2 will be initiated.

Briefly for Specific Aim 1, we will continue to evaluate methods to resolve the solubility problem of the bacterial expressed protein, such as performing RBD gene harmonization to increase the ability of bacteria cells to process and translate proteins correctly and to expand the expression refolding strategies to find optimal folding conditions that could lead to a soluble bacteria expressed recombinant RBD protein. In addition, we will continue to further study the yeast expression/fermentation patterns of RBD193-N3, RBD219-N1 and RBD219-N3 and we will extend the initial work to optimize the fermentation and purification of rRBD proteins and improve yield, purity and stability. Immunogenicity and antigenicity will continue to be evaluated in parallel to the production efforts. Detection of the antibody and cellular immune responses induced by rRBD proteins after vaccination in the presence or absence of GLA (Glycopyranosyl lipid A) and/or alum adjuvants will continue followed by the evaluation of cross-neutralizing activity with a SARS-CoV pseudovirus neutralization assay. We have been in close contact with our industrial partner ImmuneDesign. They are providing valuable technical advice and we have executed a Material Transfer Agreement to obtain the necessary adjuvants. These experiments will include the optimization of the immunization regimens, such as adjuvant formulation (GLA-AF, GLA-SE, or alum), antigen doses (1, 5, 10, or 20 µg), and administration number and interval of immunizations. Mouse sera, spleens and lymph nodes will be collected 10 days after last boost for detection of the RBD-specific antibody and T cell immune responses and neutralization as described above. Finally, we will test the protective efficacy of rRBD proteins against lethal challenge with either homologous or heterologous strains of viruses. We will continue to closely coordinate with the consortium partners NYBC and UTMB to fully execute the year 2 studies.

B.2 Year 1 Accomplishments. For this reporting period the major activities were linked to **Specific Aim 1:** Expression, purification and pre-clinical characterization of the rRBD protein as a vaccine candidate. Overall yeast expression revealed that at least one of our constructs (rRBD193-N1) shows great promise for production and scale-up (up to 0.5 g/L yields). Briefly, two receptor-binding domain (RBD) gene sequences of the SARS-CoV spike (S) protein, named RBD193 and RBD 219 (Du L et al., *Viral Immunol.* 2010 Apr;23(2):211-9.; He Y et al., *Vaccine* 2006;24:5498–508; Du L et al., *Virology* 2009;393:144-50), were cloned into two protein expression systems, *E. coli* (bacteria) and *Pichia pastoris* (yeast). The bacterial-expressed and yeast-expressed proteins were produced and evaluated based on **1A. Feasibility of scalable expression** - yield, scalability, purity and preliminary stability profile, **1B. Antigenicity** - recognition of recombinant (r) RBD protein by conformation-dependent monoclonal antibodies (mAbs), **1C. Immunogenicity** - ability of the rRBD protein to elicit RBD-specific humoral and cellular immune responses and **1D. Efficacy** - obtain and expand various strains of SARS-CoV to be used for challenge studies.

Specific Aim 1.A. Feasibility of scalable expression.

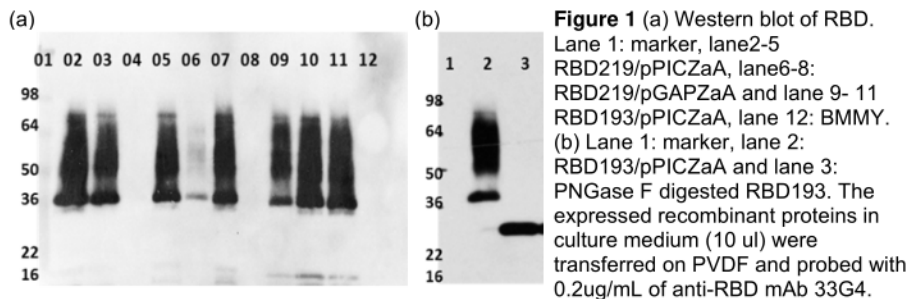
1.A.1. *E. coli* (bacteria) Expression. The wild type (wt) RBD193 and RBD219 gene sequences were codon optimized, cloned and expressed in the pET/BL21 system. Evaluation of the proteins expressed at the shaker flask level showed that high yield expression could be observed but the rRBD protein was insoluble and found in the inclusion bodies (data not shown). To increase the solubility of these bacterial expressed rRBD proteins, we optimized various parameters including: changes in IPTG concentration; different expression temperatures (16-42°C); differences in the induction time (1 hr to overnight); and modified the cell lysis methods. Additional strains of *E. coli* were also evaluated such as BL21-RIPL; Rosetta2 pLysS; and Origami2 pLysS. None of these optimization strategies nor the usage of additional bacterial strains helped increase the solubility of the expressed rRBD protein. Future proposed experiments are described in Section B6 of this report.

1.A.2. *Pichia pastoris* (yeast) Expression. We evaluated the yeast expression system since it is well proven to overcome production hurdles traditionally observed with the *E. coli* system. Briefly, the wt RBD193 and RBD219 codon optimized genes were engineered and cloned into two yeast expression vectors, pPICZαA and pGAPZαA. Proteins were initially expressed in shaker flasks and results showed good feasibility of soluble rRBD proteins expressed in the pPICZαA system. This system was selected for further evaluation.

1.A.3. Protein yield, purity, and preliminary stability profile.

Protein yield. As stated above, initial shaker flask results showed good predicted protein yields based on visible bands on an SDS-PAGE Coomassie stained gels (data not shown). This meets our internal criteria to proceed and evaluate protein expression in fermenters rather than shaker flask. However, after initial purity and stability evaluation by Western immunoblot analysis results indicate that the wtRBD proteins are either heavily glycosylated or aggregated (Figure 1a); high degree of glycosylation of the RBD protein was confirmed by digesting the protein with N-glycosidase PNGase F (Figure 1b). This finding could potentially limit our ability to have an accurate and reproducible control during scale-up production and quality control testing. No evidence of aggregation was observed indicating a promising stability profile. In parallel we evaluated the yields expressing the wt RBD193 clone at the 5L fermentation scale; results showed that the wt RBD193 protein during the fermentation process had a toxic effect on the host yeast cell viability and predicted protein yields were not achieved. This finding would impede reaching the required yields needed for large-scale production. Due to this finding we considered a more detailed assessment of the cloning strategy by using gene deletion and mutagenesis strategies (see below).

Preliminary stability profile. To evaluate further the high degree of glycosylation observed and to determine if this post-translational modification could be involved in the toxicity during protein production, the deletion and/or site-directed mutagenesis of the RBD predicted N-linked glycosylation sites was conducted serially. The deletion/mutagenesis produced three different and new inserted RBD genes into the vector pPICZαA for both RBD193 and RBD219: a) N1: removal of 1st glycosylation site; N2: removal of the 1st site and mutation of the 2nd site; and, N3: removal of the 1st site and mutation of the 2nd and 3rd sites. The N1, N2 and N3 yeast-expressed recombinant proteins for RBD193 and RBD219 were evaluated and compared based on expression patterns and yields. Results show that the removal and mutation of the N-linked glycosylation sites facilitated



the production of a soluble macromolecule that can be detected as a distinct and discrete band on a western blot probed with a conformational epitope-specific mAb 33G4 (Figure 2). These data are encouraging because it would allow us to have accurate and reproducible control during scale-up production and quality control testing. However, we also observed that successive de-glycosylation decreased the levels (yield) of expression ($N1 > N2 > N3$). This suggests the necessity to strike a balance between yield and quality control to produce a discrete protein product.

Yield and purity of RBD193N1. A fermentation run at the 5L scale of the clone RBD193N1 showed that the deletion of the sequences encoding for the first N-glycosylation site resolved the issue of toxicity and restored expected yeast cell viability. After the 5L fermentation run a yield of 400 mg/L total expressed recombinant protein was estimated based on densitometry (Figure 3a). Initial attempts to purify rRBD193-N1 from the fermented culture using hydrophobic interaction chromatography and subsequent size exclusion column allowed us to obtain 95% of purified protein evidenced both by SDS-PAGE Coomassie stained gels and Western blot analysis (Figure 3b). These are very promising results and will allow us to continue to focus our work on the yeast expression system for further process development.

1.B. Antigenicity. The rRBD193-N1 was evaluated by Western blot analysis (Figure 4A) with a series of RBD-specific mAbs (He Y et al., *Vaccine* 2006;24:5498–508; Du L et al., *Virology* 2009;393:144-50). A mammalian 293T cell-expressing wtRBD193 was used as positive control (He Y et al., *J. Immunol.* 2005; 174:4908–15). Results show that the rRBD193-N1 protein (5 μ g/well) could be recognized by all the tested conformational epitope-specific mAbs and linear epitope-specific mAbs (0.33 μ g/ml), with the strongest reaction observed in Conf V mAb 33G4 and the linear mAb 17H9. By ELISA (Figure 4B), results show that rRBD193-N1 (1 μ g/ml) could also react with all tested mAbs (3.33 μ g/ml), although its reactivity with Conf I mAb 24H8 and Conf VI mAb 19B2 is lower than that of wtRBD193. Both assays show consistent results indicating that rRBD193-N1 protein maintains conformation and antigenicity despite deletion of the N1 glycosylation site.

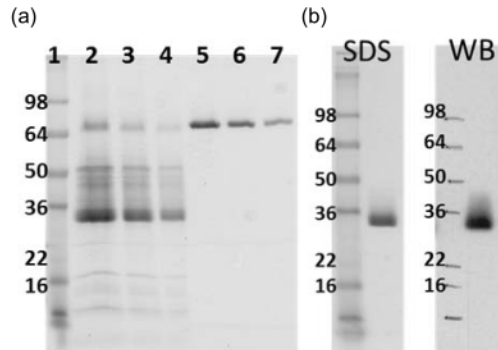
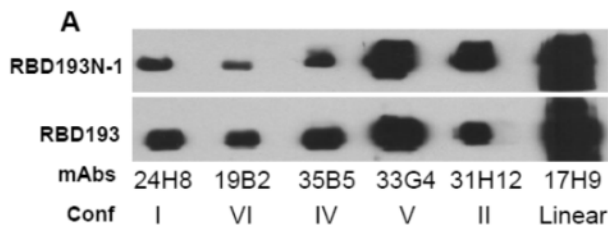


Figure 3 (a) SDS PAGE of RBD193-N1 and BSA standard. Lane 1: marker, 10 μ L, 5 μ L and 2.5 μ L of RBD193-N1 in lane 2, 3 and 4 respectively followed by 900ng, 450ng and 225ng of BSA in lane 5, 6 and 7 respectively. (b) SDS PAGE (left figure) and western blot (right figure) of 2mg purified RBD 193-N1. Noted that western blot was probed with 0.2 μ g/mL of anti-RBD mAb 33G4.

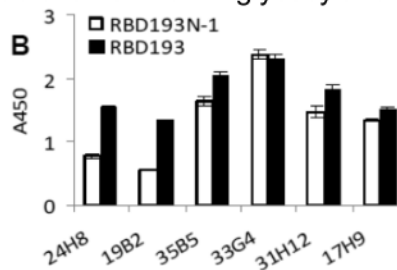


Figure 4. Antigenicity and functionality analysis of purified RBD193N-1 protein. Reactivity of RBD193N-1 protein with mAbs specific for the conformational and linear epitopes of RBD by Western blot (A) and ELISA (B), respectively. Wild-type SARS RBD193 protein was included as the positive control. The data in (B) are expressed as the mean \pm standard deviation (SD) of duplicate wells.

1C. Immunogenicity. The rRBD193-N1 protein was used to immunize 6-8 week-old BALB/c mice for three times at 3-week intervals after adsorption to Alhydrogel® adjuvant (InvivoGen, CA). The wtRBD193 (mammalian) protein and PBS were used as positive and placebo controls, respectively. Sera were collected before immunization and 10 days post-each vaccination. Determination of the anti-RBD antibody titers, their neutralizing capability and the isotypes are ongoing.

1D. Efficacy. We have obtained and expanded various strains of SARS-CoV. Clinical or recombinant isolates were generated by reverse genetics, some of which were mouse-adapted (MA). Unlike the transgenic mouse model where lethality of SARS-CoV-infected animals involved severe pathological lesions of both lungs and brains, the wt mice infected by MA virus appear to die of pulmonary pathology with minimal, if any, of CNS involvement. The newly established stocks of MA viruses are ready to test the efficacy of rRBD proteins to protect against heterologous challenge (i.e., cross protection) in wt mice.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Jiang S, Bottazzi ME, Du L, Lustigman S, Tseng CT, Curti E, Jones K, Zhan B, Hotez PJ. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. Expert Rev Vaccines. 2012 Dec;11(12):1405-13. PubMed PMID: 23252385; PubMed Central PMCID: PMC3586247.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING**C.5.a Other products**

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	HOTEZ, PETER,	PII		BA,PHD, MD	PD/PI	EFFORT	0	0			NA
	Y	JIANG, SHIBO,			PHD,MD	PD/PI		0	0			NA
	N	Tao, Xinrong,			Ph.D.	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0	0			NA
	Y	Zhan, Bin,			MD	Co-Investigator		0	0			NA
	N	Hudspeth, Elissa,			BS	Technician		0	0			NA
	N	Seid, Chris,			Ph.D.	Staff scientist (Doctoral level)		0	0			NA
	N	Chag, Shivali,			MS	Non-Student Research Assistant		0	0			NA
	N	Chen, Wen,			Ph.D.	Non-Student Research Assistant		0	0			NA
	N	Tricoche, Nancy,			BS	Non-Student Research Assistant		0	0			NA
eRA Commons User Name	N	Ma, Cuiqing,			Ph.D	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position		0	0			NA
	Y	Chan, Tehseng,	MD, PhD	Co-Investigator	0	0			NA			
eRA Commons User Name	Y	Du, Lanying,	PHD	Co-Investigator	0	0			NA			
	Y	Tseng, Chien-Te,	PHD,MS	Co-Investigator	0	0			NA			
	Y	Bottazzi, Maria,	PHD	PD/PI	0	0			NA			
	Y	LUSTIGMAN, SARA,	PHD	Co-Investigator	0	0			NA			
	N	Nino, Diane,	BSci	Project Manager	0	0			NA			

Glossary of acronyms:

S/K - Senior/Key
 DOB - Date of Birth
 Cal - Person Months (Calendar)
 Aca - Person Months (Academic)
 Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation
 SS - Supplement Support
 RE - Reentry Supplement
 DI - Diversity Supplement
 OT - Other
 NA - Not Applicable

D.2 PERSONNEL UPDATES**D.2.a Level of Effort**

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

NOTHING TO REPORT

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

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D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

NOTHING TO REPORT

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

NOTHING TO REPORT